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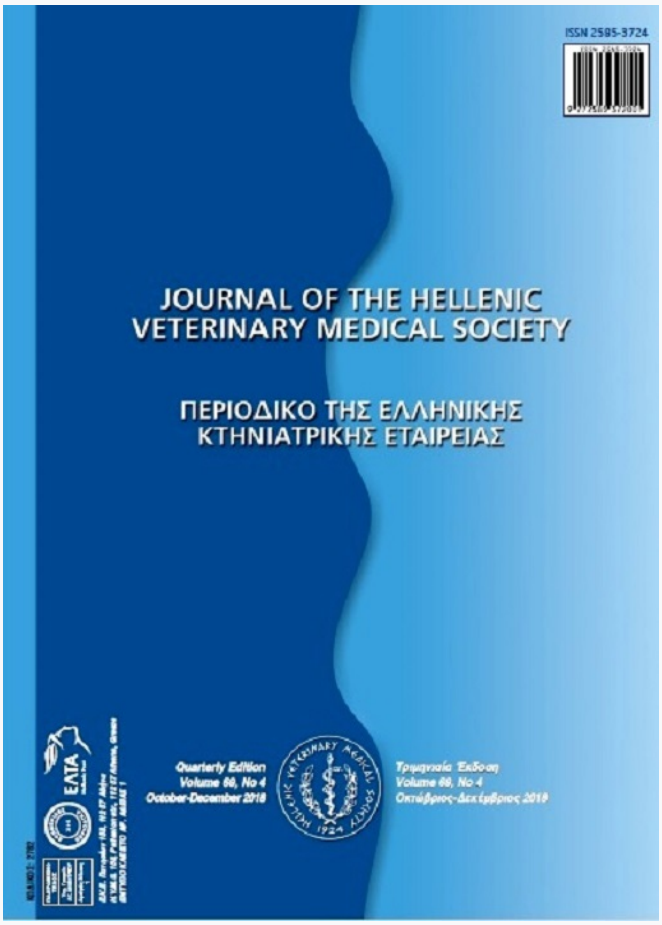

Πρώτη αναφορά του Angiostrongylus vasorum σε κόκκινες αλεπούδες (Vulpes vulpes) στη Σερβία

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Research article
Ερευνητικό άρθρο

First report of *Angiostrongylus vasorum* in red foxes (*Vulpes vulpes*) in Serbia

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ABSTRACT. Angiostrongylosis caused by *Angiostrongylus vasorum* is an emerging disease in Europe and the red fox (*Vulpes vulpes*) is considered as a main reservoir species for this parasite. Since there have been no reports of *A. vasorum* in red foxes in Serbia at the time of carrying out our investigations, the aim of the investigations was to explore the role of red foxes in South Banat (northern Serbia) as reservoirs for *A. vasorum*. Legally hunted foxes were autopsied in the Veterinary Specialised Institute "Pančevo". The heart, lungs and pulmonary artery were examined macroscopically for evidence of gross lesions and for the presence of adult specimens of *A. vasorum*. Impression smears of the changed lung tissue were examined microscopically for the presence of first stage larvae of *A. vasorum* and histopathological examination was performed on lung samples. Out of 24 examined foxes hunted in different locations, 13 had lesions manifested in the lungs, which were suspected to be indicative of angiostrongylosis. In the majority of the foxes distal parts of the pulmonary lobes were swollen, firm, and discoloured to dark-red, dark-yellow and dark-brown. The characteristic lesions in distal parts of the pulmonary lobes were completely consistent with the presence of adult parasites in the right heart and pulmonary arteries, and with the presence of the first stage larvae in the impresion smears. The present finding contributes to the knowledge of geographic distribution of angiostrongylosis in red foxes in Europe and provides valuable information that should raise awareness in veterinarians to consider this parasitosis in dogs with signs of cardiopulmonary diseases.

Keywords: *Angiostrongylus vasorum*, angiostrongylosis, red foxes, Serbia

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INTRODUCTION

Angiostrongylosis caused by metastrongyloid nematode *Angiostrongylus vasorum* (the French heartworm) is an emerging disease in Europe (Traversa et al., 2010). *Angiostrongylus vasorum* has an indirect life cycle, residing in its adult form in the pulmonary arteries and right heart of domestic dogs (*Canis lupus familiaris*) and wild carnivores of the family Canidae, in particular the red fox (*Vulpes vulpes*), recognized as an important reservoir of the parasite (Bolt et al., 1994).

The oviparous female lays eggs that hatch to the first-stage larvae (L1). The L1 penetrate into the alveoli and are coughed up, swallowed and excreted into the environment with the feces, where they can potentially infect intermediate hosts. The intermediate hosts are snails and slugs, in which the L1 larvae develop to the third-stage larvae (L3), infective for definitive and paratenic (transport) hosts (Guilhon, 1963; Morgan et al., 2005; Koch and Willesen, 2009; Elsheikha et al., 2014). Definitive hosts acquire the infection through ingestion of an intermediate or paratenic host or through ingestion of free L3 present in the environment (Elsheikha et al., 2014).

Although *A. vasorum* was discovered for the first time in 1853 (Serres, 1854), reports of the disease in Europe have been increasing only over the past three decades. Possible reasons should be found in the growing awareness among practitioners and researchers, and in the increased prevalence of the parasite (Traversa et al., 2010). These first reports of angiostrongylosis in red foxes from European countries came from France, Ireland, Switzerland, and Italy (Poli et al., 1984). Currently, *A. vasorum* has a worldwide distribution, although it usually maintains in small enzootic foci (Helm et al., 2010). Canine angiostrongylosis is considered endemic in certain areas of Europe, including regions of Denmark, Germany, Hungary, Finland, France, Ireland, Italy, the Netherlands, Poland, Slovakia, Spain, Sweden, Switzerland, Turkey and the United Kingdom (Elsheikha et al., 2014).

The distribution of *A. vasorum* in red foxes in Europe is well investigated in some countries (Saeed et al., 2006; Morgan et al., 2008; Schug et al., 2013; Eleni et al., 2014; Härtwig et al., 2015; Santoro et al., 2015; Tolnai et al., 2015; McCarthy et al., 2016) while on the other hand, there are still many uninvestigated regions. At the time of carrying out our investigations, the disease was reported in Serbia in a dog in the region of Srem (Simin et al., 2014) and in a golden jackal in the region of South Banat (Gavrilović et al., 2017). Since there have been no reports of *A. vasorum* in red foxes, the aim of the investigations was to explore their role as reservoirs for *A. vasorum* in two selected hunting grounds in South Banat (northern Serbia).

MATERIAL AND METHODS

The investigation area

The investigations were conducted in the area around the City of Pančevo (100,000 inhabitants), located at the confluence of the Tamiš and Danube rivers, and only 17 km distant from Belgrade, the capital of Serbia. Pančevo is the administrative center of South Banat, one of the Administrative Districts of Vojvodina Province. A northern part of Serbia was chosen as the target area because in previous investigations carried out in the Scientific Veterinary Institute of Serbia, during a two year period from 2015 to 2017, 192 specimens of red foxes, golden jackals and wolves from central and southern Serbia tested negative for the presence of *A. vasorum* (Pavlović, unpublished data).

Animals

Twenty four foxes legally hunted at different locations around the City of Pančevo during a three month period from December 2016 to February 2017 were delivered to the Veterinary Specialised Institute "Pančevo", the Department of Pathology and Parasitology within the Programme for Control and Eradication of Rabies in Serbia. The foxes originated from two hunting grounds: Nadel, whose area is 38,682.47 ha and Stari Tamiš, with an area of 33,211.23 ha. Twelve foxes from each hunting ground were presented for examination.

Methods

Twenty four foxes were necropsied and the heart, pulmonary artery and lungs were examined macroscopically for evidence of gross lesions and the presence of adult specimens of *A. vasorum*. Impression smears of the macroscopically changed lung tissue were examined microscopically for the presence of the first stage larvae of *A. vasorum*. The adult parasites and first stage larvae were identified on the basis of the characteristic

morphological features described by Guilhon and Cens (1973) and McGarry and Morgan (2009), respectively.

Macroscopically changed lungs were sampled for histopathology. For histopathological examination tissue samples were fixed in 10% buffered formalin. After processing in an automated tissue processor, the samples were embedded in paraffin blocks. Approximately 5 μm thick paraffin sections were stained using the standard haematoxylin-eosin (HE) method.

RESULTS

Of the 24 examined foxes, hunted in different locations around Pančevo, in 13 of them lesions suspected to be indicative of angiostrongylosis were found in the lungs. Distal parts of the pulmonary lobes were swollen, firm, and discoloured to dark-red, dark-yellow and dark-brown and occasionally with whitish areas (fibrosis). The cut surface of the changed lung tissue was dry and granular. In the majority of the foxes lesions were seen in the periphery of the lung lobes, while in one fox the majority of the right diaphragmatic lobe was grossly affected (Fig. 1a).

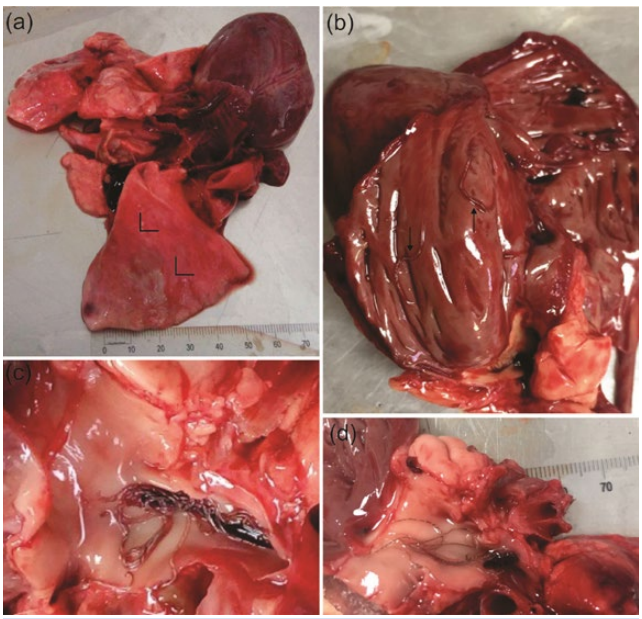

Fig 1: Gross lesions in the lungs and the presence of adult *Angiostrongylus vasorum* in the heart and pulmonary artery: (a) The majority of the right diaphragmatic lung lobe is swollen and discoloured; (b) Two adult parasites in the right heart chamber; (c) Numerous adults of *A. vasorum* in the pulmonary artery curled up around each other; (d) Adult females in the pulmonary artery recognized by the "barber pole" appearance

Fig 2: A first stage larva of *Angiostrongylus vasorum* from the lungs of an infected fox

Adult parasites were found in the right ventricle (Fig. 1b) and pulmonary arteries (Fig. 1c, d) in all the 13 foxes that had gross lesions in the lungs. In the other foxes, in which the lungs were without gross lesions, no adult parasite was found in the right heart or pulmonary arteries. Microscopy of impression smears of the changed lung tissue found the first stage larvae of *A. vasorum* (Fig. 2).

Histopathology revealed parasitic larvae and eggs in the lung tissue (Fig. 3). Histopathological lesions included vascular damage, haemorrhage, haemosiderosis, multifocal granulomas, generation of fibrous tissue in the alveolar septa, and hyperplasia and hypertrophy of the *tunica muscularis* of the arteries containing adult parasites.

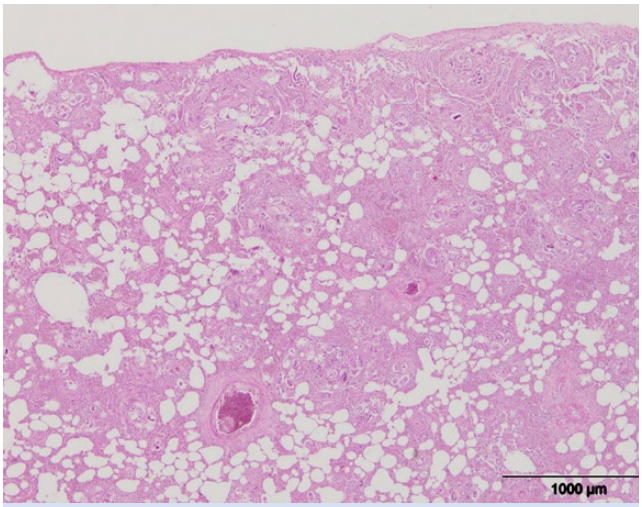

Fig 3: Histopathological lesions in the lungs of a fox infected with *Angiostrongylus vasorum*. Eggs and larvae of the parasite in the lung tissue (HE staining)

DISCUSSION

The present finding is, to the best of our knowledge, the first report of enzootic angiostrongylosis in red foxes in Serbia. The disease was diagnosed in the majority of foxes from both investigated hunting grounds. As the agent was found in 13 of the 24 examined foxes (54.17%) the results indicate a high prevalence of the infection in the area of investigation.

In countries neighboring Serbia, a prevalence of 17.9% has been reported in the fox population in Hungary (Tolnai et al., 2015), and less than 5% in the fox population in Croatia (Rajkovic-Janje et al., 2002). The overall prevalence of 7.3% has been reported in the UK fox population varying widely between regions, from 0% in Scotland and northern England to 23% in south-east England (Morgan et al., 2008). In the Republic of Ireland the incidence of infection was found to be 39.9% with positive samples occurring in each of the country's 26 counties (McCarthy et al., 2016). In Denmark, recognized as an endemic area, *A. vasorum* was recorded in 48.6% of foxes (Saeed et al., 2006). The disease has recently been diagnosed in German red foxes, showing regionally differing prevalences of 8.4 %, 19.1 %, 27.3%, and 9% for Thuringia, Hesse, Rhineland-Palatine, and the Federal State of Brandenburg, respectively (Schug et al., 2013; Härtwig et al., 2015). In Portugal, *A. vasorum* was found in 16.1% of 62 red foxes (Eira et al., 2006). A prevalence of 23.88% has been found in red foxes in Tuscany (Poli et al., 1984), 43.5% in Central Italy (Eleni et al., 2014), 33.3% in southern Italy (Santoro et al., 2015), and 78.2% in north-west Italy (Magi et al., 2015) . Prevalence in red foxes in Poland in the Forest District Głęboki Brod in Augustowska Primeval Forest has been estimated at 5.2% (Demiaszkiewicz et al., 2014). The prevalence was estimated based on necropsy and examination of the heart and lungs in all the studies except for the study by Härtwig et al. (2015) who used real-time PCR.

Pathomorphological lesions are comparable to those described by Poli et al. (1984) who found in the lungs large wedge-shaped areas of reddish-brown coloration with increased consistency. Similarly, Santoro et al. (2015) described reddish-brown and yellow-brown foci with increased resistance to slicing and scattered areas of emphysema. Eleni et al. (2014) found severe pulmonary lesions in 25.9% of examined dead foxes in Italy. In three foxes, almost the whole diaphragmatic lobes have been consolidated, similar to our finding for one fox in which the majority of the right diaphragmatic lobe was consolidated. Unlike the present study, the samples originated from dead animals in which the lesions would have been much more pronounced.

The red fox is the natural reservoir of *A. vasorum*, capable of sustaining the infection without serious clinical consequences (Eleni et al., 2014). However, considering the severe gross lesions in the lungs (Fig 1a) and the high level invasion in some of the animals examined in the present study (Fig 1c), angiostrongylosis should be considered as a disease that may affect the health status of the red fox population. Such an opinion is supported by Eleni et al. (2014) who found remarkable gross lesions in the lungs of dead foxes in Italy and, at least for three specimens, angiostrongylosis was considered the cause of death.

Out of 24 foxes examined in this study, 13 were found to have the first stage larvae of *A. vasorum* in the lungs which implies that they would have excreted larvae and transmitted the infection to their habitats. The Baermann's diagnostic method which would prove this statement was not used in the study. As the red fox frequently comes close to cities and urban areas in which there are dogs, there is a high probability for this parasitosis to be transmitted to dogs in which the infection can be fatal (Benda et al., 2017). Dogs from Pančevo and nearby places including Belgrade - the capital of Serbia - should, therefore, be considered under the high risk from angiostrongylosis. Stray dogs and hunting dogs visiting the habitats of red foxes are under a higher risk of infection.

Pathomorphological findings observed in the lungs of infected foxes show that the infection has a chronic course, and confirm the role of the red fox as an important reservoir and transmitter of angiostrongylosis in the target area of northern Serbia. Until 2013, when angiostrongylosis was diagnosed in a dog from the region of Srem, there had been no data related to the presence of *A. vasorum* in Serbia (Simin et al., 2014). Considering the high prevalence in red foxes as the main reservoir species in the area of investigation, it may be assumed that the disease is prevalent and neglected in Serbia. A lack of awareness among practitioners in Serbia of the presence of the agent could have resulted in past misdiagnosis of the disease in dogs. Such an opinion is supported by the findings of Schnyder et al. (2015) who

found 1.36 % *Angiostrongylus* antibody- and antigen-positive dogs in Hungary, bordering Serbia in north. On the contrary, some authors believe that relatively high level of pathogenicity associated with *A. vasorum* infection makes it unlikely that clinical cases of the disease have been missed in the past years (Traversa et al., 2010). Based on the present investigations it is very possible for the disease to have existed in Serbia, at least in red foxes, for many years before the first case was diagnosed in a dog in 2013.

The trend towards a higher incidence of angiostrongylosis observed in some European countries and the concomitant expansion in foxes strongly indicate that the parasite is actually emerging in Europe (Morgan et al., 2009; Helm et al., 2010; Traversa et al., 2010). Recently, the first case of aelurostrongylosis in a kitten was reported in the investigation area (Gavrilović et al., 2017). This is also a gastropod-borne disease with a similar life cycle that completes in the cat as the definitive host. The finding of aelurostrongylosis in a cat, coupled with the finding of angiostrongylosis in red foxes, leads to the conclusion that gastropod-borne parasitoses are emerging in South Banat which seems to provide suitable conditions for the completion of the life cycle of both *A. abstrusus* and *A. vasorum*. The life cycle and the dynamics and activity of the population of gastropod intermediate hosts are mainly influenced by temperature, moisture and water availability (Traversa et al., 2010; Traversa and Di Cesare, 2014).

CONCLUDING REMARKS

The present investigations confirmed that the target area in South Banat, northern Serbia should be considered as an enzootic focus of angiostrongylosis, contributing to the knowledge of geographic distribution of the disease in red foxes in Europe. The paper provides strong evidence for veterinarians to consider angiostrongylosis in dogs with signs of cardiopulmonary disease in Serbia and other uninvestigated countries. Further investigations should be carried out in order to assess the true prevalence of *A. vasorum* in red foxes, other wild canids and domestic dogs in Serbia.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Benda T, Csivincsik Á, Nemes C, Turbók J, Zsolnai A, Simonyai E, Majoros G, Nagy G (2017) Lethal Angiostrongylus vasorum infection in a Hungarian dog. Acta Parasitol 62: 221-224.

Bolt G, Monrad J, Koch J, Jensen AL (1994) Canine angiostrongylosis: a review. Vet Rec 135: 447-452

Demiaszkiewicz AW, Pyziel AM, Kuligowska I, Lachowicz J (2014) The first report of *Angiostrongylus vasorum* (Nematoda; Metastrongyloidea) in Poland, in red foxes (*Vulpes vulpes*). Acta Parasitol 59: 758-762.

Eleni C, Grifoni G, Di Egidio A, Meoli R, De Liberato C (2014) Pathological findings of Angiostrongylus vasorum infection in red foxes (Vulpes vulpes) from Central Italy, with the first report of a disseminated infection in this host species. Paras Res 113: 1247-1250.

Eira C, Vingada J, Torres J, Miquel J (2006) The helminth community of the red fox, *Vulpes vulpes* in Dunas de Mira (Portugal) and its effect on host condition. Wildl Biol Pract 2: 26-36.

Elsheikha HM, Holmes SA, Wright I, Morgan ER, Lacher DW (2014) Recent advances in the epidemiology, clinical and diagnostic features, and control of canine cardiopulmonary angiostrongylosis. Vet Res 45: 92.

Gavrilović P, Jovanović M, Gavrilović A, Nešić S (2017) Fatal aelurostrongylosis in a kitten in Serbia. Acta Parasitol 62: 488-491.

Gavrilović P, Marinković D, Todorović I, Gavrilović A (2017) First report of pneumonia caused by *Angiostrongylus vasorum* in a golden jackal. Acta Parasitol 62: 880-884.

Guilhon J (1963) Recherches sur le cycle évolutif du strongle des vaisseaux du chien. Bull Acad Vet 36: 431-442.

Guilhon J, Cens B (1973) Angiostrongylus vasorum (Baillet. 1866): étude biologique et morphologique. Ann Parasitol Hum Comp 48: 567-596.

Härtwig V, Schulze C, Barutzki D, Schaper R, Daugschies A, Dyachenko V (2015) Detection of Angiostrongylus vasorum in Red Foxes (Vulpes vulpes) from Brandenburg, Germany. Parasitol Res 114: 185-92.

Helm JR, Morgan ER, Jackson MW, Wotton P, Bell R (2010) Canine angiostrongylosis: an emerging disease in Europe. J Vet Emerg Crit Care 20: 98-109.

Koch J, Willesen JL (2009) Canine pulmonary angiostrongylosis: an update. Vet J 179: 348-359

Magi M, Guardone L, Prati M, Mignone W, Macchioni F (2015) Extraintestinal nematodes of the red fox Vulpes vulpes in northwest Italy. J Helminthol 89: 506-511.

McCarthy G, Ferrand M, De Waal T, Zintl A, McGrath G, Byrne W, O'neill EJ (2016) Geographical distribution of Angiostrongylus vasorum in foxes (Vulpes vulpes) in the Republic of Ireland. Parasitology 143: 588-593.

McGarry JW, Morgan ER (2009) Identification of first-stage larvae of metastrongyles from dogs. Vet Rec 165: 258-26.

Morgan ER, Jefferies R, Krajewski M, Ward P, Shaw SE (2009) Canine pulmonary angiostrongylosis: the influence of climate on parasite distribution. Parasitol Int 58: 406-410.

Morgan ER, Shaw SE, Brennan SF, De Waal TD, Jones BR, Mulcahy G (2005) *Angiostrongylus vasorum*: a real heartbreaker. Trends Parasitol 21: 49-51.

Morgan ER, Tomlinson A, Hunter S, Nichols T, Roberts E, Fox MT, Taylor MA (2008) Angiostrongylus vasorum and Eucoleus aerophilus in foxes (Vulpes vulpes) in Great Britain. Vet Parasitol 154: 48-57.

Poli A, Arispici M, Marconcini A, Mancianti F, de Monte D (1984) Angiostrongylus vasorum (Baillet, 1866) in Red Foxes (*Vulpes vulpes* L.) in Italy. J Wildl Dis 20: 345-346.

Rajković-Janje R, Marinculić A, Bosnić S, Benić M, Vinković B, Mihaljević Ž (2002) Prevalence and seasonal distribution of helminth parasites in red foxes (*Vulpes vulpes*) from Zagreb County (Croatia). Z Jagdwiss 48: 151-160.

Santoro M, D'Alessio N, Di Prisco F, Neola B, Restucci B, Pagano TB, Veneziano V (2015) Angiostrongylus vasorum infection in red foxes (Vulpes vulpes) in southern Italy. Acta Parasitol 60: 356-359.

Saeed I, Maddox-Hyttel C, Monard J, Kapel CMO (2006) Helminths of red foxes *(Vulpes vulpes)* in Denmark. Vet Parasitol 139: 168-179.

Schnyder M, Schaper R, Lukács Z, Hornok S, Farkas R (2015) Combined serological detection of circulating *Angiostrongylus vasorum* antigen and parasite-specific antibodies in dogs from Hungary. Parasitol Res 114: 145-54.

Schug K, Hermosilla C, Hirzmann J, Schaper R, Taubert A (2013) Endoparasitenfauna beim Rotfuchs in Deutschland unter besonderer Berücksichtigung von *Angiostrongylus vasorum*. Tagung DVG-Fachgruppe "Parasitologie und Parasitäre Erkrankungen" Gießen, 8.-10. July 2013: 79-80.

Serres E (1854) Entozoaires trouvés dans l'oreille droite, le ventricule correspondant et l'artère pulmonaire d'un chien. J Vét Midi 7: 70.

Simin S, Kosić LS, Kuruca L, Pavlović I, Savović M, Lalošević V (2014) Moving the boundaries to the South-East: first record of autochthonous *Angiostrongylus vasorum* **infection in a dog in Vojvodina province, northern Serbia.** Parasit Vectors 7: 396-402.

Tolnai Z, Széll Z, Sréter T (2015) Environmental determinants of the spatial distribution of Angiostrongylus vasorum, Crenosoma vulpis and Eucoleus aerophilus in Hungary. Vet Parasitol 207: 355-358.

Traversa D, Di Cesare A (2014) Cardio-pulmonary parasitic nematodes affecting cats in Europe unraveling the past, depicting the present, and predicting the future. Front Vet Sci 1: 11.

Traversa D, Di Cesare A, Conboy G (2010) Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. Parasit Vectors 3: 62.